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OMB APPROVED
0579-0036

This report is required by law (7 U.S.C. 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

Interagency Report Control
No. 0180-DOA-AN

Fiscal Year: 2009

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

REGISTRATION NUMBER: 13-R-0014

Customer Number: 45318

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA, include ZIP Code)

Calamouni Research & Development Company
P O Box 1100
Saint Albans, VT 05478

Telephone: (802) 527 2364

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ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS (Sites) See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A.)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be attached to this report.)	F. TOTAL NUMBER OF ANIMALS (Cols. C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs		15		160	175
7. Hamsters					
8. Rabbits					
9. Non-human Primates					
10. Sheep					
11. Pigs					
12. Other Farm Animals					
13. Other Animals					

ASSURANCE STATEMENTS

- 1.) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.
- 2.) Each principal investigator has considered alternatives to painful procedures.
- 3.) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- 4.) The attending veterinarian for this research facility has appropriate authority to ensure the provisions of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer (C.E.O.) or Legally Responsible Institutional Official (L.R.O.))
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

DATE SIGNED

11/8/09

(b)(6), (b)(7)c

56 12-4-09

Column E Explanations**Registration number:** 13-R-0014**STUDY 1****Number of animals used in this study:** 18**Species of animals used in this study:** Guinea Pig**Explain the procedure producing pain or distress.**

The objective of the current study is to specifically characterize a guinea pig model of stress/anxiety. This study involves a small group of animals to evaluate the proper shock threshold for stressing these animals without causing unnecessary harm or ill effects. The appropriate shock level was then used in behavioral studies in order to evaluate each method's efficacy in evaluating the level of stress/anxiety in the guinea pig

Foot-Shock -On the first day of testing the animals will be brought into the lab and acclimated for 1 hour. Following acclimation subjects will undergo 15 trials of 3 second, 0.5-1.5mA footshock. On the second day the subjects will be returned to the same context where shock occurred previously. Responses to the environment as well as fecal output will be monitored.

Fear Potentiated Startle- The procedure involves subjecting the animals to 20 tone/shock pairs (80dB 5000hZ tone, 1.0mA foot-shock) in the startle apparatus. 48 hours later animals are again placed into the startle chambers and subjected to noise burst only, and tone + noise burst (100dB) trials. Augmentation of the startle response in tone + noise burst trials can be attributed to increased anxiety driven by the prior tone/shock pairings.

Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

The following table summarizes the number of citations found during our Pubmed Literature Search

Number of citations obtained from Pubmed using the designated Keyword Combinations			
	Footshock	Fear Potentiated Startle	Anxiety
Guinea Pig	6	2	165
Guinea Pig AND Alternative	0	0	2

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Guinea pigs have not been used to a significant degree in studies of fear or stress/anxiety. The most common use of guinea pigs in such studies is the so-called "mother-pup separation" paradigm. In these studies, guinea pig pups are separated from their mothers, and vocalizations made by the pups are recorded. Pups show increased vocalizations when separated from their mothers, and this response is sensitive to anxiolytic compounds in some studies. Footshocks have been used in a few studies to elicit fear/stress/anxiety responses in guinea pigs. The 2 citations showing FPS and Guinea Pigs did not test FPS in guinea pigs, rather guinea pigs were used in other aspects of the studies.

The objective of the current study is to specifically characterize a guinea pig model of stress/anxiety. Such a model could then be used in studies examining the effects of chronic stress/anxiety on aspects of physiological function such as gastrointestinal motility, where guinea pig models of gut function are well established.

In order to study fear/stress/anxiety in an animal model, there must be an aversive situation for the animal, such as the foot shock, for them to develop a fear and that fear must be sustained in order to test it. The animal will remember and develop an association of the place in which it happened and other cues that are associated with that aversive situation. When the animal is exposed to those cues (whether it be a tone or a light) the animal makes a response (such as potentiated startle) that is taken to indicate a fearful state due to the memory of the aversive situation (e.g. foot shock). There are no less-invasive alternative approaches to developing a state of fear and then testing for its presence than with the startle apparatus.

It should be noted that animals in a fear potentiated startle protocol do not generally show any overt signs of being stressed or in pain/distress, other than vocalizing/jumping during the brief footshocks during conditioning.

The goal of this study is to utilize small groups of animals to evaluate the proper shock threshold for stressing these animals without causing unnecessary harm or ill effects. The appropriate shock level was then used in freeze and startle testing in order to evaluate each methods efficacy in evaluating the level of stress/anxiety in the guinea pig.

Column E Explanations

Registration number:

13-R-0014

STUDY 2

Number of animals used in this study:

20

Species of animals used in this study:

Guinea Pig

Explain the procedure producing pain or distress.

Our goal is to establish a guinea pig model for examining stress or anxiety-induced bowel dysfunction. This protocol utilizes multiple procedures that are designed to induce stress in the guinea pig. The stressors used will be (a) forced swim, (b) pedestal, (c) tonic immobility, (d) fear potentiated startle and (e) foot-shock.

- (a) The forced swim test will utilize a six-minute swimming period in room temperature water (22 deg. C).
- (b) The pedestal treatment places the animal on a small, brightly lit, elevated platform for 30 minutes.
- (c) The tonic immobility test involves inducing the animals final escape response, lack of motion. Animals will be inverted and held against the laboratory bench until they no longer struggle (maximum time of 60 seconds).
- (d) The fear potentiated startle procedure involves subjecting the animals to 20 tone/shock pairs (80dB 5000Hz tone, 1.0mA foot-shock) in the startle chambers. 24 hours later animals are again placed into the startle chambers and subjected to noise burst only, and tone + noise burst (100dB) trials. Augmentation of the startle response in tone + noise burst trials can be attributed to increased anxiety driven by the prior tone/shock pairings.
- (e) The foot-shock stressor will involve 10, 3-second, mild foot-shocks (1 mA) in a conditioning chamber.

Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Irritable Bowel Syndrome (IBS) is a disorder that effects the gastrointestinal motility in the form of diarrhea or constipation. IBS symptoms may be triggered by stressful events. Our goal is to establish a guinea pig model for examining stress or anxiety-induced bowel dysfunction. In vitro and in vivo methods for characterizing bowel function are well established in the guinea pig. However, very little research has been done examining behavioral models of stress/anxiety in this species. Recent research has implicated the Corticotropin Releasing Factor (CRF) pathway in the brain, and altered serotonin signaling in the gut as major causes of the symptoms of IBS including altered motility and visceral sensation. Providing the guinea pig with stressful events should activate the central CRF pathway causing an alteration in fecal pellet motility peripherally. If successful, this work would result in a novel model facilitating the development of treatments for IBS that could improve quality of life for individuals with IBS. Stressful events are known to bring about the pathology seen in irritable bowel syndrome. A method for producing these types of events in the guinea pig is necessarily stressful. Since the goal of this study is to examine bowel function in stressed animals compared to unstressed animals, the stressed animals necessarily must undergo treatments that will result in stress.

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Column E Explanations**Registration number:**13-R-0014**STUDY 3****Number of animals used in this study:**122**Species of animals used in this study:**Guinea Pig**Explain the procedure producing pain or distress.**

The goal of this study is to characterize a model of stress/anxiety in the guinea pig. We will use a procedure that is designed to induce a state of fear in the guinea pigs in response to a normally non-stressful audible tone. Animals will be trained to be afraid of tone (80dB 5000Hz) by pairing it with an mild and very brief footshock (250ms 1.0mA foot-shock). During the training session animals will receive 20 tone/shock pairs in a startle chamber, following a 5 min acclimation period in which the animals are allowed to become comfortable in the test environment. 48 hours after training animals will be tested in the same chamber. After a 5 min acclimation period, the test involves a short noise burst (100dB white noise) that acts as a startle stimulus. The startle stimulus is presented alone, or paired with the same tone used during fear training. 10 trials of each type (stimulus alone, or tone + stimulus) are presented following a 10 trial leader period (stimulus alone). The difference in startle amplitude between stimulus alone and tone + stimulus trials is a measure of fear.

Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

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Guinea pigs have not been used to a significant degree in studies of fear or stress/anxiety. The most common use of guinea pigs in such studies is the so-called "mother-pup separation" paradigm. In these studies, guinea pig pups are separated from their mothers, and vocalizations made by the pups are recorded. Pups show increased vocalizations when separated from their mothers, and this response is sensitive to anxiolytic compounds in some studies. Footshocks have been used in a few studies to elicit fear/stress/anxiety responses in guinea pigs. The 2 citations showing FPS and Guinea Pigs did not test FPS in guinea pigs, rather guinea pigs were used in other aspects of the studies.

The objective of the current study is to specifically characterize a guinea pig model of stress/anxiety. Such a model could then be used in studies examining the effects of chronic stress/anxiety on aspects of physiological function such as gastrointestinal motility, where guinea pig models of gut function are well established.

In order to study fear/stress/anxiety in an animal model, there must be an aversive situation for the animal, such as the foot shock, for them to develop a fear and that fear must be sustained in order to test it. The animal will remember and develop an association of the place in which it happened and other cues that are associated with that aversive situation. When the animal is exposed to those cues (whether it be a tone or a light) the animal makes a response (such as potentiated startle) that is taken to indicate a fearful state due to the memory of the aversive situation (e.g. foot shock). There are no less-invasive alternative approaches to developing a state of fear and then testing for its presence than with the startle apparatus.

It should be noted that animals in a fear potentiated startle protocol do not generally show any overt signs of being stressed or in pain/distress, other than vocalizing/jumping during the brief footshocks during conditioning.

ANIMAL USE SUMMARY:

STUDY 1: 18 Animals
 STUDY 2: 20 Animals
 STUDY 3: 122 Animals

TOTAL ANIMALS USED: 160 Animals

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